

## Data Evaluation Report on the Chronic Toxicity of Metconazole to Freshwater Invertebrates - *Daphnia* sp.

EPA MRID No. 47795005

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<b>Data Requirement:</b>	EPA DP Barcode	371496
	EPA MRID	47795005
	EPA Guideline	850.1300

**Test material:** Metconazole, technical-grade      **Purity:** 99.4% (84.1% *cis* isomer, 15.3% *trans* isomer)  
**Common name** Metconazole  
**Chemical name:** IUPAC: (1*RS*,5*RS*;1*RS*,5*SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)-cyclopentanol  
CAS name: 5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol  
CAS No.: 125116-23-6  
Synonyms: KNF-S-474m

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**Date:** 01/07/11 *Alicia Korol 1/13/11*

**EPA PC Code** 125619

**Date Evaluation Completed:** 01/07/11

**CITATION:** Sayers, L.E. 2009. Metconazole – Full Life-Cycle Toxicity Test with Water Fleas (*Daphnia Magna*) Under Static-Renewal Conditions, Following OPPTS Draft Guideline 850.1300. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA. Laboratory Study No. 12709.6275. Study sponsored by Valent U.S.A., Walnut Creek, CA. Study initiated July 29, 2008 and completed January 21, 2009.

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**EXECUTIVE SUMMARY:**

The 21-day-chronic toxicity of metconazole to *Daphnia magna* was studied under static-renewal conditions. Daphnids were exposed to metconazole at nominal concentrations of 0 (negative control), 0.016, 0.031, 0.063, 0.13, 0.25, and 0.50 mg ai/L. Mean-measured concentrations were <0.00050 (<LOQ, control), 0.017, 0.031, 0.060, 0.12, 0.23, and 0.48 mg ai/L, respectively. The 21-day EC<sub>50</sub> for adult immobilization was >0.48 mg ai/L, the highest concentration tested. The 21-day NOAEC, based upon treatment-related reductions in total length (the most sensitive endpoint) at the ≥0.060 mg ai/L levels, was 0.031 mg ai/L.

Parental survival averaged 90 to 100% for all levels (including control), with no treatment-related effect observed. The first brood release occurred on Days 8 for the control through 0.12 mg ai/L levels, and on Day 9 for the 0.23 and 0.48 mg ai/L levels. Offspring production was statistically-reduced (p<0.05) at the ≥0.12 mg ai/L levels; the mean number of live offspring released per daphnia was 177, 177, 189, 161, 134, 121, and 91 for the control, 0.017, 0.031, 0.060, 0.12, 0.23, and 0.48 mg ai/L levels, respectively. There were no immobilized offspring reported.

Terminal body length of surviving first-generation daphnia (the most sensitive endpoint) averaged 5.16, 5.17, 5.08, 5.05, 4.94, 4.95, and 4.78 mm for the control, 0.017, 0.031, 0.060, 0.12, 0.23, and 0.48 mg ai/L levels, respectively. Differences were statistically-reduced (p<0.05) compared to the control at the ≥0.060 mg ai/L levels. Mean dry weight of surviving first-generation daphnids was 1.17, 1.12, 0.99, 1.16, 1.31, 1.22, and 0.89 mg, respectively. The difference was statistically-reduced (p<0.05) compared to the control and treatment groups only at the 0.48 mg ai/L level.

This study is scientifically sound and meets OCSPP 850.1300 guideline standards for a chronic toxicity test on freshwater invertebrates using *Daphnia magna*. **This study is classified Acceptable.**

**Results Synopsis**

Test Organism Age (eg. 1<sup>st</sup> instar): First instar, <24 hours old  
Test Type (Flow-through, Static, Static Renewal): Static-renewal

Parental Survival

EC<sub>50</sub>: >0.48 mg ai/L                      95% C.I.: N/A  
NOAEC: 0.48 mg ai/L  
LOAEC: >0.48 mg ai/L

Reproduction (mean offspring/parent)

NOAEC: 0.060 mg ai/L  
LOAEC: 0.12 mg ai/L

Total Length Parents

NOAEC: 0.031 mg ai/L  
LOAEC: 0.060 mg ai/L

Dry Weight Parents

NOAEC: 0.23 mg ai/L  
LOAEC: 0.48 mg ai/L

Endpoints affected: offspring production, total length, and dry weight  
Most sensitive endpoint(s): total length

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## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** U.S. EPA OCSPP (form. OPPTS) 850.1300 (*draft*, 1996)

1. OCSPP guidance states that among replicate test chambers of a given level, the measured concentration of the test material should not vary more than 20%. In this study, analytical variation ranged from 32 to 34% for all levels.
2. The test temperature deviated from  $20\pm 1^{\circ}\text{C}$  on Days 14, 15, and 21, when a minimum of  $18^{\circ}\text{C}$  was recorded.

These deviations do not affect the scientific soundness or acceptability of this study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in accordance with GLP Standards as published in 40 CFR Part 160 with the following exceptions: routine water and food contaminant screening analyses.

### A. MATERIALS:

**1. Test Material** Metconazole technical-grade

**Description:** Not reported

**Lot No./Batch No. :** AS2122a

**Purity:** 99.4% (84.1% *cis* isomer, 15.3% *trans* isomer)

**Stability of compound under test conditions:** In general, concentrations were satisfactorily maintained within 20% of the mean-measured values for all treatment levels. However, at the nominal 0.031, 0.13, and 0.50 mg ai/L levels, single results per level exceeded  $\pm 20\%$  of the mean-measured concentrations, and at the nominal 0.063 mg ai/L level, two results exceeded  $\pm 20\%$  of the mean-measured concentration. In all cases, the outliers were  $+20\%$  of mean-measured concentrations in freshly-prepared media (on Days 2 or 5).

**Storage conditions of test chemicals:** In the original container at  $-10$  to  $-25^{\circ}\text{C}$

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**Physicochemical properties of metconazole.**

Parameter	Values	Comments
Water solubility at 20°C	17.1 mg/L cis-isomer 13.6 mg/L trans-isomer	Temp. not reported (source: MRID 46902213)
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

*(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)*

**2. Test Organism:**

**Species:** *Daphnia magna*, first instar, <24 hours old  
*EPA and OECD recommend Daphnia magna*

**Age of the parental stock:** Not reported  
*EPA recommends that young daphnids #24 hours old from a separate parental culture be used*

**Source:** Laboratory culture  
*EPA requires all test organisms must be produced from laboratory reared culture that has been maintained for at least 21 days at test conditions in dilution water with renewal of the culture medium at least three times per week.*

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding Study: A 21-day exploratory range-finding study was performed under static-renewal conditions by exposing daphnid neonates (<24 hours old) to nominal concentrations of 0 (negative control), 0.0050, 0.050, 0.50, and 5.0 mg ai/L. There were five replicates per level, with one daphnid per replicate vessel. Test solutions were clear and colorless, with no undissolved test substance evident. After 21 days, survival was 100% in control organisms, compared to 80, 100, 0, and 0% at the 0.0050, 0.050, 0.50, and 5.0 mg ai/L levels, respectively. The number of offspring per parent averaged 200, 193, 180, 23, and 0 for the control, 0.0050, 0.050, 0.50, and 5.0 mg ai/L levels, respectively. Total lengths of surviving daphnids exposed at 0.0050 and 0.050 mg ai/L averaged 4.78 and 4.58 mm, respectively, compared to 4.93 mm for the control animals. Nominal concentrations selected for use in the definitive study were based upon these results and consultation with the Sponsor.

b. Definitive Study

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**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Parental acclimation:</u> Period:  Conditions: (same as test or not)  Feeding:          Health (any mortality observed):	Continuous  Same as test  Daily with a combination of a unicellular green algae, ( <i>Ankistrodesmus falcatus</i> , $4 \times 10^7$ cells/mL), and a suspension of yeast, cereal leaves and digested flaked fish food (YCT): at 0 to 6 days old – 1.0 mL of algae and 0.5 mL of YCT suspension per vessel per day; at 7 to 10 days old – 1.5 mL of algae and 0.5 mL of YCT suspension per vessel per day; and >10 days old – 2.0 mL of algae and 0.5 mL of YCT suspension per vessel per day.  0% immobilization 48 hours prior to test initiation	Brood daphnia were maintained in dilution water at $20 \pm 2^\circ\text{C}$ under a 16-hour light photoperiod (76 to 93 footcandles at the surface of the culture solution).  The adult daphnia used to supply offspring did not contain ephippia, were 7 days old when offspring were first produced, produced an average of five offspring per female over a 7-day period prior to test initiation, and were not used in any portion of a previous test.  <i>EPA recommends that prior to testing, daphnids that are at least 10-12 days old (those that have had at least one brood) should be separated from the culture, put in separate container and maintained for at least 21 days to insure that good health conditions are present</i>
<u>Test condition:</u> static renewal/flow-through:  Type of dilution system- for flow through method.  Renewal rate for static renewal	Static-renewal  N/A  At test initiation and at 48- or 72-hour intervals.	  <i>(EPA requires consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)</i>
Aeration, if any	None reported.	  <i>EPA recommends test chambers should not be aerated</i>

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Parameter	Details	Remarks
		Criteria
Duration of the test	21 days	Recommended duration is 21 days.
<u>Test vessel</u> Material: (glass/stainless steel)  Size (for growth and reproduction/survival test):  Fill volume:	Glass  100 mL  80 mL	1. <u>Recommended Material:</u> Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <u>Recommended Size:</u> 250 ml with 200 ml fill volume; 100 ml with 80 ml fill volume OECD guideline recommends that parent animals be maintained individually; one per vessel, with 50 - 100 ml of medium in each vessel.
Source of dilution water	Culture and dilution water were prepared in 1900-L batches by fortifying well water according to the formula for hard water (U.S. EPA, 1975). The prepared water was passed through an Amberlite® XAD-7 resin column to remove any potential organic contaminants. Water quality parameters were measured on each batch prior to use; the prepared water had a total hardness range of 160 to 170 mg/L as CaCO <sub>3</sub> , alkalinity range of 100 to 110 mg/L as CaCO <sub>3</sub> , pH range of 7.8 to 8.5, a dissolved oxygen (DO) range of 8.4 to 9.9 mg/L, and a specific conductance range of 550 to 650 µmhos/cm.	Fortified water was discarded if not used within 14 days.  Recommended source of dilution water includes unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).

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Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u> Hardness  pH  Dissolved oxygen  Temperature  Total Organic Carbon Particulate matter Inorganic analytes Pesticides Chlorine  Frequency of measurements	170 to 180 mg/L as CaCO <sub>3</sub>  7.8 to 8.7  7.7 to 11 mg/L (>60% saturation)  18 to 21°C  0.35 mg/L (Aug. 2008) Not reported Not reported Not reported Not reported  DO, temperature, and pH were measured in each solution at the beginning (new) and end (aged) of each renewal period; aged solutions consisted of a composite sample of all ten replicates per level. Temperature was also continuously monitored in the water bath. Total hardness, alkalinity, and specific conductance were measured in the freshly-prepared solution of the control and highest level (0.50 mg ai/L)	Alkalinity was 100 to 110 mg/L as CaCO <sub>3</sub> and specific conductance was 500 to 650 µmhos/cm.  During the first 7 days of exposure, DO levels were <i>ca.</i> 120% saturation in several of the aged solutions. The increase was attributed to the presence of food algae that the neonate daphnids were unable to remove from the test solutions during this time.  <hr/> <b>Recommended hardness:</b> 160 to 180 mg/L as CaCO <sub>3</sub> ; OECD recommends >140 mg/L as CaCO <sub>3</sub> <b>Recommended pH:</b> 7.6 to 8.0 pH should not deviate by more than 1.0 unit for more than 48 hours. OECD recommends that pH range be 6 - 9 and does not vary more than 1.5 units in any one test. <b>Recommended dissolved oxygen:</b> renewal should not drop below 50% for more than 48 hours. Recommended flow-through: ≥60% throughout test. <b>Recommended temperature:</b> 20°C ± 2°C.; should not deviate from 20°C by more than 5°C for more than 48 hours. OECD recommends a range of 18 - 22°C; temperature should not vary more than ± 2°C OECD guideline recommends that total organic carbon <2 mg/L

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Parameter	Details	Remarks
		Criteria
<u>Number of replicates:</u> For growth and reproduction:  For survival test:	10 replicates per level  Same	There were six toxicant levels spaced by a separation factor of 2.
		<i>Number of replicates should include a control(s) and at least 5 test concentrations; dilution factor should not be greater than 50%.            OECD recommends that at least 5 test concentrations be used in a geometric series with a separation factor not exceeding 3.2.</i>
<u>Number of organisms:</u> For growth and reproduction:  For survival test:	1 organism per replicate  Same	Each replicate vessel (with 80 mL of solution) contained 1 daphnia, for a loading rate of 0.012 daphnia/mL solution. OPPTS guidance reports that loading should not exceed 40 daphnia per L solution in the static-renewal systems (equivalent to $\leq 0.04$ daphnia/mL).
		<i>Recommended number of organisms include 22 daphnids/test concentration; 7 test chambers should contain 1 daphnid each, and 3 test chambers contain 5 daphnids each.            OECD recommends holding a minimum of 10 daphnids individually for static tests.            For flow-through tests, 40 animals should be divided into 4 groups of 10 animals at each test concentration.</i>

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Parameter	Details	Remarks
		Criteria
<u>Treatment Concentrations:</u> nominal:  mean-measured:	0 (negative control), 0.016, 0.031, 0.063, 0.13, 0.25, and 0.50 mg ai/L  <0.00050 (<LOQ, control), 0.017, 0.031, 0.060, 0.12, 0.23, and 0.48 mg ai/L	Water samples were collected from freshly-prepared test solutions prior to allocation into the test vessels on Days 0, 2, 5, 9, 14, and 19, and from a composite sample of aged test solutions created from all replicates (per level) on Days 2, 5, 9, 14, 19, and 21.  Analytical variation (high to low) was excessive, and ranged from 32 to 34% for all levels.  Mean-measured concentrations were 92 to 100% of nominal levels.
Solvent (type, percentage, if used)	N/A	<i>Solvent concentration should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD recommends #0.1 ml/L of solvent.</i>
Lighting	16-hours light, 8-hours dark, avoiding sudden transitions. The intensity ranged from 11 to 15 $\mu\text{Em}^{-2}\text{S}^{-1}$ during exposure.	Intensity was inadvertently not reported in terms of footcandles.  <i>Recommended photoperiod is 16 hours light and 8 hours of dark.</i>
Recovery of chemical:  Frequency of measurement:  LOD: LOQ:	76.9 to 114% of nominal (excluding two outliers of 140 and 156%)  Days 0, 2, 5, 9, 14, 19, and 21  Not reported 0.00050 mg ai/L	Based on QC samples fortified at 0.00800, 0.100, or .500 mg ai/L and analyzed concurrently with the test samples (total of 21 samples).
Positive control {if used, indicate the chemical and concentrations}	None tested	

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Parameter	Details	Remarks
		Criteria
Other parameters, if any Feeding:	During the test, daphnids were fed once daily with 200 µL of a unicellular green algae, ( <i>Ankistrodesmus falcatus</i> , 4 x 10 <sup>7</sup> cells/mL), and 50 µL of a suspension of yeast, cereal leaves and digested flaked fish food (YCT).	

## **2. Observations:**

**Table 2: Observations**

Parameters	Details	Remarks
		Criteria
Data endpoints measured (list)	<ul style="list-style-type: none"> <li>- Parental immobility</li> <li>- Other parental sub-lethal effects</li> <li>- Time to first brood release</li> <li>- Offspring production</li> <li>- Offspring immobility</li> <li>- Terminal length and dry weight of surviving P generation</li> </ul>	<p><i>Recommended endpoints measured:</i></p> <ul style="list-style-type: none"> <li>- Survival of first-generation daphnids,</li> <li>- Number of young produced per female,</li> <li>- Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test,</li> <li>- Observations of other effects or clinical signs.</li> </ul>
Observation intervals	All test vessels were examined daily. Growth measurements were determined on Day 21.	
Were raw data included?	Yes	
Other observations, if any	The physical characteristics of the test solutions were recorded daily.	

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**II. RESULTS AND DISCUSSION**

**A. MORTALITY:**

No treatment-related effect on survival (i.e., immobility) was observed, with no statistically-significant differences from the control indicated for any level. Parental survival averaged 90 to 100% for all levels (including control). The 21-day LC<sub>50</sub> was estimated to be >0.48 mg ai/L, the highest mean-measured concentration tested. The NOAEC for parental survival was 0.48 mg ai/L.

**Table 3: Effect of Metconazole on Survival, Reproduction, and Growth of Daphnia sp.**

Treatment Mean-measured (and nominal) conc. mg a.i./L	Cumulative Mortality (dead or immobile)		Mean Day of First Brood	Mean No. Young Per Surviving Adult	Mean Total Body Length, mm ± SD	Mean Dry Weight, mg ± SD
	No.	%				
Control (dilution water only)	0	0	8	177	5.16 ± 0.11	1.17 ± 0.12
0.017 (0.016)	1	10	8	177	5.17 ± 0.13	1.12 ± 0.21
0.031 (0.031)	0	0	8	189	5.08 ± 0.10	0.99 ± 0.18
0.060 (0.063)	0	0	8	161	5.05 ± 0.08*	1.16 ± 0.18
0.12 (0.13)	1	10	8	134*	4.94 ± 0.10*	1.31 ± 0.08
0.23 (0.25)	0	0	9	121*	4.95 ± 0.08*	1.22 ± 0.14
0.48 (0.50)	0	0	9	91*	4.78 ± 0.10*	0.89 ± 0.10*
NOAEC, mg ai/L	0.48		0.060		0.031	0.23
LOAEC, mg ai/L	>0.48		0.12		0.060	0.48
LC/EC <sub>50</sub> (with 95% C.I.), mg ai/L	>0.48		Not reported	Not reported	Not reported	Not reported

\*Statistically-reduced compared to the control (Williams' Test, p<0.05).

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**B. EFFECTS ON REPRODUCTION AND GROWTH:**

The first brood release occurred on Day 8 for the control through (mean-measured) 0.12 mg ai/L levels, and on Day 9 for the 0.23 and 0.48 mg ai/L levels. The mean number of live offspring released per daphnia was 177, 177, 189, 161, 134, 121, and 91 for the control, 0.017, 0.031, 0.060, 0.12, 0.23, and 0.48 mg ai/L levels, respectively. Differences were statistically-reduced ( $p < 0.05$ ) compared to the control at the  $\geq 0.12$  mg ai/L levels. Therefore, the NOAEC for reproduction was 0.060 mg ai/L, and the LOAEC was 0.12 mg ai/L.

Apparently, there were no immobilized offspring produced.

Terminal body length of surviving first-generation daphnia was the most sensitive endpoint. Mean total body lengths were 5.16, 5.17, 5.08, 5.05, 4.94, 4.95, and 4.78 mm for the control, 0.017, 0.031, 0.060, 0.12, 0.23, and 0.48 mg ai/L levels, respectively. Differences were statistically-reduced ( $p < 0.05$ ) compared to the control at the  $\geq 0.060$  mg ai/L levels. Mean dry weight of surviving first-generation daphnids was 1.17, 1.12, 0.99, 1.16, 1.31, 1.22, and 0.89 mg, respectively. Dry weights were statistically-reduced ( $p < 0.05$ ) compared to the control only at the 0.48 mg ai/L level. Based on terminal lengths, the NOAEC for growth was 0.031 mg ai/L.

**C. REPORTED STATISTICS:**

Data that were statistically analyzed included organism survival (i.e., immobilization), reproduction (cumulative number of offspring produced per female), and growth (as total body length and dry body weight). The time to first brood release data were not statistically assessed. Offspring produced by adults that died during the test were excluded.

Survival data were analyzed first using fisher's Exact Test, and any level indicating significant effects were excluded from further statistical analyses. Reproduction and growth data were checked for normality using the Chi-Square Test and for homogeneity of variance using Bartlett's Test. All data met these assumptions and were therefore analyzed using (the parametric) Williams' Test. All analyses were conducted at the 95% level of certainty except in the case of the Chi-Square and Bartlett's Tests, in which the 99% level of certainty was applied.

The NOAEC and LOAEC were based on significance data. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. All analyses were performed using TOXSTAT® version 3.5 and mean-measured concentrations.

During this study, no concentration caused a reduction of  $\geq 50\%$  immobilization; therefore, the 21-day  $EC_{50}$  was empirically estimated to be greater than the highest mean-measured concentration.

Parental Survival

LC<sub>50</sub>:  $>0.48$  mg ai/L                      95% C.I.: N/A  
NOAEC: 0.48 mg ai/L  
LOAEC:  $>0.48$  mg ai/L

Reproduction (offspring/parent)

NOAEC: 0.060 mg ai/L  
LOAEC: 0.12 mg ai/L

Total Length

NOAEC: 0.031 mg ai/L  
LOAEC: 0.060 mg ai/L  
MATC (geometric mean): 0.043 mg ai/L

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## Dry Weight

NOAEC: 0.23 mg ai/L

LOAEC: 0.48 mg ai/L

## **D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method(s): The reviewer statistically verified results for survival, reproduction (offspring produced per daphnid), length, and dry weight. Reproduction and growth data were confirmed to be normally distributed (using the Chi-square and Shapiro Wilks tests) and the variances homogeneous (using Hartley and Bartlett's tests). The NOAEC values for reproduction, length and weight were determined using ANOVA, followed by William's test. The control group survival data were compared to the treatment groups experiencing immobilized daphnia using Fisher's Exact Test. These analyses were conducted using Toxstat statistical software.

## Parental Survival

LC<sub>50</sub>: >0.48 mg ai/L

95% C.I.: N/A

NOAEC: 0.48 mg ai/L

LOAEC: >0.48 mg ai/L

## Reproduction (offspring/parent)

NOAEC: 0.060 mg ai/L

LOAEC: 0.12 mg ai/L

## Total Length

NOAEC: 0.031 mg ai/L

LOAEC: 0.060 mg ai/L

## Dry Weight

NOAEC: 0.23 mg ai/L

LOAEC: 0.48 mg ai/L

## **E. STUDY DEFICIENCIES:**

There were no study deficiencies.

## **F. REVIEWER'S COMMENTS:**

The reviewer agrees with the results obtained by the study authors.

All validity requirements were fulfilled. Specifically, control immobility was 0% (no more than 20% permissible), each control daphnid living the full 21 days produced an average of 177 young (minimum of 60 required), and no ephippia were produced by control animals.

As concentrations were relatively constant throughout the exposure period, the reviewer did not calculate time-weighted average concentrations.

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Fresh stock solutions were prepared at each renewal interval. A 5.0 mg ai/L primary stock was prepared at each interval by adding approximately 0.0100 g (0.0099 g ai) of metconazole to 2.0 L of dilution water. The stock solution was sonicated for 10 minutes, and mixed overnight using a magnetic stirrer. The primary stock was clear and colorless, with no visible un-dissolved test substance. Test solutions were then prepared by diluting the appropriate volume of the primary stock to 1.5 L with dilution water. Each test solution was stirred with a glass rod for approximately 1 minute prior to allocation into the replicate vessels. All test solutions were clear and colorless with no visible un-dissolved test substance present.

Water samples were extracted with hexane:ethyl acetate (4:1, v:v). The extracts were evaporated to dryness using a rotary evaporator at 40°C. Residues were re-constituted in toluene, vortexed for 30 seconds and sonicated for 5 minutes. Aliquots were analyzed for *cis*- and *trans*-metconazole using gas chromatography with nitrogen-phosphorus detection (GC/NPD). The method was validated in June 2008. Artificial seawater was fortified with metconazole at 0.0200 and 0.200 mg ai/L. Recoveries averaged  $103 \pm 4.48\%$ , with a LOQ of 0.000500 mg ai/L.

The experimental phase of the definitive study was conducted from August 6 to 27, 2008.

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## G. CONCLUSIONS:

This study is scientifically sound and meets OCSPP 850.1300 guideline standards for a chronic toxicity test on freshwater invertebrates using *Daphnia magna*. **This study is classified Acceptable.** Based upon treatment-related reductions in total lengths of surviving first-generation daphnia (the most sensitive endpoint), the NOAEC and LOAEC were 0.060 and 0.12 mg ai/L, respectively. The number of offspring produced per surviving adult was reduced at the  $\geq 0.12$  mg ai/L levels, and the dry weights of surviving first-generation daphnia were reduced at the 0.48 mg ai/L level. No treatment-related effect on survival of first-generation daphnia was observed. The 21-day EC<sub>50</sub> (for immobility) was  $>0.48$  mg ai/L, the highest concentration tested.

### Parental Survival

EC<sub>50</sub>:  $>0.48$  mg ai/L                      95% C.I.: N/A  
NOAEC: 0.48 mg ai/L  
LOAEC:  $>0.48$  mg ai/L

### Reproduction (offspring/parent)

NOAEC: 0.060 mg ai/L  
LOAEC: 0.12 mg ai/L

### Total Length

NOAEC: 0.031 mg ai/L  
LOAEC: 0.060 mg ai/L

### Dry Weight

NOAEC: 0.23 mg ai/L  
LOAEC: 0.48 mg ai/L

Endpoints affected: offspring production, total length, and dry weight  
Most sensitive endpoint(s): total length

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## APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.556	16.456	25.976	16.456	4.556
OBSERVED	7	13	22	26	0

Calculated Chi-Square goodness of fit test statistic = 12.7367

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

Shapiro-Wilks test for normality

\*\*\*\*\* Shapiro-Wilks Test is aborted \*\*\*\*\*

This test can not be performed because total number of replicates is greater than 50.

Total number of replicates = 68

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 5.84

Closest, conservative, Table H statistic = 13.1 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 9  
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 8.71  
(average df used)

Data PASS homogeneity test. Continue analysis.

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NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----  
Calculated B statistic = 12.09  
Table Chi-square value = 16.81 (alpha = 0.01)  
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 8.71  
Used for Chi-square table value ==> df (#groups-1) = 6  
-----

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

## ANOVA TABLE

-----  
SOURCE DF SS MS F  
-----  
Between 6 75847.365 12641.228 12.000  
Within (Error) 61 64257.856 1053.407  
-----  
Total 67 140105.221  
-----

Critical F value = 2.25 (0.05,6,60)  
Since F > Critical F REJECT Ho:All groups equal

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment  
-----  
GROUP IDENTIFICATION TRANSFORMED MEAN CALCULATED IN  
MEAN ORIGINAL UNITS T STAT SIG  
-----  
1 control 177.400 177.400  
-----

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2	0.017	176.667	176.667	0.049
3	0.031	189.300	189.300	-0.820
4	0.060	160.700	160.700	1.151
5	0.12	133.778	133.778	2.925 *
6	0.23	120.800	120.800	3.899 *
7	0.48	91.300	91.300	5.932 *

-----

Bonferroni T table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	10			
2	0.017	9	36.730	20.7	0.733
3	0.031	10	35.750	20.2	-11.900
4	0.060	10	35.750	20.2	16.700
5	0.12	9	36.730	20.7	43.622
6	0.23	10	35.750	20.2	56.600
7	0.48	10	35.750	20.2	86.100

-----

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	177.400	177.400	181.276
2	0.017	9	176.667	176.667	181.276
3	0.031	10	189.300	189.300	181.276
4	0.060	10	160.700	160.700	160.700
5	0.12	9	133.778	133.778	133.778
6	0.23	10	120.800	120.800	120.800
7	0.48	10	91.300	91.300	91.300

-----

Offspring per daphnid

File: 5005r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

**Data Evaluation Report on the Chronic Toxicity of Metconazole to Freshwater Invertebrates -  
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control	181.276				
0.017	181.276	0.260		1.67	k= 1, v=61
0.031	181.276	0.267		1.75	k= 2, v=61
0.060	160.700	1.151		1.77	k= 3, v=61
0.12	133.778	2.925	*	1.78	k= 4, v=61
0.23	120.800	3.899	*	1.79	k= 5, v=61
0.48	91.300	5.932	*	1.79	k= 6, v=61

s = 32.456

Note: df used for table values are approximate when v > 20.

Body Length

File: 50051

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.556	16.456	25.976	16.456	4.556
OBSERVED	7	12	24	24	1

Calculated Chi-Square goodness of fit test statistic = 8.9019

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Body Length

File: 50051

Transform: NO TRANSFORMATION

Shapiro-Wilks test for normality

\*\*\*\*\* Shapiro-Wilks Test is aborted \*\*\*\*\*

This test can not be performed because total number of replicates  
is greater than 50.

Total number of replicates = 68

Body Length

File: 50051

Transform: NO TRANSFORMATION

**Data Evaluation Report on the Chronic Toxicity of Metconazole to Freshwater Invertebrates -  
Daphnia sp.**

EPA MRID No. 47795005

Hartley test for homogeneity of variance

-----  
Calculated H statistic (max Var/min Var) = 2.56  
Closest, conservative, Table H statistic = 13.1 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 9  
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 8.71  
(average df used)

-----  
Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal  
but do not differ greatly, the Hartley test may still be used  
as an approximate test (average df are used).

Body Length

File: 50051 Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----  
Calculated B statistic = 2.54  
Table Chi-square value = 16.81 (alpha = 0.01)  
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 8.71  
Used for Chi-square table value ==> df (#groups-1) = 6

-----  
Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is  
used to calculate the B statistic (see above).

Body Length

File: 50051 Transform: NO TRANSFORMATION

ANOVA TABLE

-----  
SOURCE DF SS MS F  
-----  
Between 6 1.127 0.188 18.800  
Within (Error) 61 0.609 0.010  
-----  
Total 67 1.736  
-----

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Critical F value = 2.25 (0.05,6,60)  
Since F > Critical F REJECT Ho:All groups equal

Body Length  
File: 50051 Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 1 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	5.155	5.155		
2	0.017	5.167	5.167	-0.254	
3	0.031	5.080	5.080	1.677	
4	0.060	5.050	5.050	2.348	
5	0.12	4.944	4.944	4.583	*
6	0.23	4.945	4.945	4.696	*
7	0.48	4.775	4.775	8.497	*
Bonferroni T table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)					

Body Length  
File: 50051 Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 2 OF 2		Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL	
1	control	10				
2	0.017	9	0.113	2.2	-0.012	
3	0.031	10	0.110	2.1	0.075	
4	0.060	10	0.110	2.1	0.105	
5	0.12	9	0.113	2.2	0.211	
6	0.23	10	0.110	2.1	0.210	
7	0.48	10	0.110	2.1	0.380	

Body Length  
File: 50051 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	5.155	5.155	5.161
2	0.017	9	5.167	5.167	5.161
3	0.031	10	5.080	5.080	5.080
4	0.060	10	5.050	5.050	5.050

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5	0.12	9	4.944	4.944	4.945
6	0.23	10	4.945	4.945	4.945
7	0.48	10	4.775	4.775	4.775

Body Length

File: 50051

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	5.161				
0.017	5.161	0.120		1.67	k= 1, v=61
0.031	5.080	1.679		1.75	k= 2, v=61
0.060	5.050	2.350	*	1.77	k= 3, v=61
0.12	4.945	4.581	*	1.78	k= 4, v=61
0.23	4.945	4.706	*	1.79	k= 5, v=61
0.48	4.775	8.506	*	1.79	k= 6, v=61

s = 0.100

Note: df used for table values are approximate when v > 20.

Dry Weight

File: 5005w

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.556	16.456	25.976	16.456	4.556
OBSERVED	3	20	26	15	4

Calculated Chi-Square goodness of fit test statistic = 1.4914

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Dry Weight

File: 5005w

Transform: NO TRANSFORMATION

Shapiro-Wilks test for normality

\*\*\*\*\* Shapiro-Wilks Test is aborted \*\*\*\*\*

**Data Evaluation Report on the Chronic Toxicity of Metconazole to Freshwater Invertebrates -  
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---

This test can not be performed because total number of replicates  
is greater than 50.

Total number of replicates = 68

-----

Dry Weight

File: 5005w Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

-----

Calculated H statistic (max Var/min Var) = 6.35  
Closest, conservative, Table H statistic = 13.1 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 9  
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 8.71  
(average df used)

-----

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal  
but do not differ greatly, the Hartley test may still be used  
as an approximate test (average df are used).

Dry Weight

File: 5005w Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----

Calculated B statistic = 10.57  
Table Chi-square value = 16.81 (alpha = 0.01)  
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 8.71  
Used for Chi-square table value ==> df (#groups-1) = 6

-----

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is  
used to calculate the B statistic (see above).

Dry Weight

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File: 5005w Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1.156	0.193	8.391
Within (Error)	61	1.374	0.023	
Total	67	2.530		

Critical F value = 2.25 (0.05,6,60)  
Since F > Critical F REJECT Ho:All groups equal

Dry Weight  
File: 5005w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	1.173	1.173		
2	0.017	1.123	1.123	0.713	
3	0.031	0.990	0.990	2.698	*
4	0.060	1.157	1.157	0.236	
5	0.12	1.308	1.308	-1.934	
6	0.23	1.224	1.224	-0.752	
7	0.48	0.892	0.892	4.143	*

Bonferroni T table value = 2.46 (1 Tailed Value, P=0.05, df=60,6)

Dry Weight  
File: 5005w Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	10			
2	0.017	9	0.172	14.6	0.050
3	0.031	10	0.167	14.2	0.183
4	0.060	10	0.167	14.2	0.016
5	0.12	9	0.172	14.6	-0.135
6	0.23	10	0.167	14.2	-0.051
7	0.48	10	0.167	14.2	0.281

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---

Dry Weight  
File: 5005w

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	10	1.173	1.173	1.173
2	0.017	9	1.123	1.123	1.158
3	0.031	10	0.990	0.990	1.158
4	0.060	10	1.157	1.157	1.158
5	0.12	9	1.308	1.308	1.158
6	0.23	10	1.224	1.224	1.158
7	0.48	10	0.892	0.892	0.892

Dry Weight  
File: 5005w

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	1.173				
0.017	1.158	0.216		1.67	k= 1, v=61
0.031	1.158	0.222		1.75	k= 2, v=61
0.060	1.158	0.222		1.77	k= 3, v=61
0.12	1.158	0.216		1.78	k= 4, v=61
0.23	1.158	0.222		1.79	k= 5, v=61
0.48	0.892	4.187	*	1.79	k= 6, v=61

s = 0.150

Note: df used for table values are approximate when v > 20.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	0.017 and 0.12	10	1	